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Cryo Electron Tomography -3D imaging at nm resolution

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INTRODUCTION: Electron tomography is a technique that uses a Transmission Electron Microscope to determine a three-dimensional (3D) structure from any given asymmetric object [1]. It is the leading method for studying 3D ultrastructure in the 5-20 nm resolution range allowing complex pleomorphic assemblies, such as organelles and cellular substructures, to be studied in their cellular context. This imaging method can be simply broken down into 3 steps. First, a series of two dimension projection images of the specimen are recorded and systematically tilted to different angles (-70°/+70°) in the microscope. Second, these individual images are aligned to a common origin and finally the projections are then backprojected to create a 3D representation of the sample [2].

For high-resolution imaging techniques, such as electron tomography, there is a need to maintain the position of the cell constituents at a scale finer than the desired resolution. Cryo-fixation is a method that exploits the high content of water in biological material and uses it as a fixative. Contrary to any fixation method that relies on diffusion, cryo-fixation is fast, does not change the state of the specimen and has the potential to preserve biological structures at the atomic level. Vitrification of the sample is the ultimate goal of cryo-fixation and two cryo-fixation methods are generally used in electron tomography [3]. Plunge freezing is suitable for small protein assemblies in suspension while high-pressure freezing (HPF) has been adapted to vitrify thick samples. Plunge freezing samples can be viewed directly but HPF samples must be sectioned, either in their frozen state (cryoultramicrotomy) or freeze substituted, resin embedded and sectioned at ambient temperature.

Successful imaging of the specimen invariably depends on choosing the appropriate specimen thickness and dose rate to provide a signal to noise ratio that is high enough to produce a meaningful image. For the tilt series required for electron tomography this dose must be partitioned between the many images taken at various tilt angles. Automation in modern microscopes, by computer control, minimises the electron dose on the sample during the tilt series as it automatically corrects for image shifts and focus changes during acquisition, without subjecting the sample to additional electron exposure.

Since goniometers are not perfectly eucentric, images of the tilt series must be translated and rotationally aligned for computation of the 3D reconstruction. Once the tilt images are aligned the 3D reconstruction can be computed using a back-projection algorithm. The resulting reconstruction can be viewed as sequential thin 2D slices through the sample or the most common approach is to use segmentation to extract features of interest from the volume. In some cases individual molecules (e.g structures resolved by x-ray crystallography) can be fitted to the features in the reconstructed volume [4]. This allows the rotational orientation of molecules to be defined within the structure, making tomograms a very elegant way of displaying ultrastructure in 3D.

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